EXHIBIT
1

ANDREW GOBEA:

RECEIVED 60cc CORD BLOOD (w CPD)
PRE monos 30%
Polys 70%

added 15ml 1xPBS to bring to incubation volume of 75ml.
Added 1.5ml antibody (12.8)
incubated 25ml.
Primed CellPro "Ceprate".

Spun cells. Resuspended in 1x PBS to a final volume of 300cc in bag.

Ran through column.

Unadsorbed portion → spun down. and consolidated in 1x PBS for incubation.

75ml for incubation (added HSA)
1.5ml antibody (12.8) 25min. Spun down following incubation. Rspd to Vol. of 300cc in bag
Ran through 2nd column.

Stem cell portions from Runs 1 & 2 were combined (after initial counts done and samples removed for staining)

Total cells 2.8×10^6 for transduction

BBMM +
IL3 SANDOZ
warm
IL6 SANDOZ
warm
SCF AMGEN
warm

final concen
will be dil
cells are
concentrat

BBMM: FB
PBS

2.8×10^6 cells
Put in

BBMM + 316/SCF (for 500ml of media)

IL3 SANDOZ * Y0230892 stock at 150ug/ml
want final: 20ng/ml x 2 .. 20ug 133ul add

IL6 SANDOZ * Y0150392 stock at 150ug/ml
want final: 50ng/ml x 2 ∴ 50ug 333ul add

SCF AMGEN * 1509F2 stock at 1.5mg/ml = 1500ug/ml
want final: 100ng/ml x 2 ∴ 100ug 67ul add

final concentrations are doubled since the media will be diluted 1:2 w/ viral supernatant. Cells are therefore incubated with the correct concentrations.

BBMM: FBS Genuni LOT* A0003H
BSA *115

2.8x10^6 cells want final: 5x10^4 cells.
Put in 2 T-75 30ml each: 15ml B365 051193
15ml LAS-V^GT LOT* 53
+ protamine sulfate 240ul of 1:20 diluted stock

Cord Blood cells pre processing:

CFUs: SET 143

	Plate #	Sample	# Cells	# ul/ml media
-G418	1ab	Gabea UCB	5×10^4	50
+G418	2ab	↓	↓	50
-G418	3ab		1×10^5	100
+G418	4ab	↓	↓	100

Start:

adsorbed fraction:

adsorbed fraction

CFUs Post transduction: SET 144

	plate #	# CELLS	# ul
-G418	1ab	500	7
↓	2ab	1000	14
	3ab	2000	28
+G418	4ab	500	7
↓	5ab	1000	14
	6ab	2000	28

(yields) adsorbed

↑ count:

$\bar{x} = 34$

$\times 2 \times 10^4 = 6.8 \times 10^5$ Cfu/ml

$\times 5.5$ ml $= 3.7 \times 10^6$ C

Refused on 5/15/93

No transduced stem cells

G418			
&	7ab	1000	20
+	8ab	2000	40

adsorbed

*cell/ml media

50
50
00
100

5e+144

Start:

	PRE	Post ab
5×10^8 c	0.71%	0.22%
#34+ =	3.6×10^6 c	= 1.1×10^6 c

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adsorbed Fraction #1:

	Fl1 Fl2 gate	FL1 FSC gate
2×10^6 c	31.94%	20.81%
#34+ =	0.64×10^6 c	= 0.42×10^6 c

adsorbed Fraction #2:

0.8×10^6 c	2.46%	5.80%
#34+c =	0.02×10^6 c	0.05×10^6 c

(yields)

adsorbed #1:

PRE & FL1/FL2 gate: $\frac{0.64\times10^6 c}{3.6\times10^6 c}$ = **17.8%**

PRE & FL1/FSC gate: $\frac{0.42\times10^6 c}{3.6\times10^6 c}$ = **11.7%**

post ab & FL1/FL2 gate: $\frac{0.64\times10^6 c}{1.1\times10^6 c}$ = **58.2%**

Post ab & FL1/FSC gate: $\frac{0.42\times10^6 c}{1.1\times10^6 c}$ = **38.2%**

adsorbed #2: PRE & FL1/FL2 | PRE & FL1/FSC

~~[illegible]~~

~~[illegible]~~

ZACHARY RIGGINS 5/14/93

RECD 200cc CORD BLOOD

PRE: mono 109 ✓ poly 109

218 x 50 = 10.9 x 10^6 c/ml
x 200ml = 2.2 x 10^9 C start

Added 3 vials (4.5ml) 12.8 ab
inc. 25 min.

Spun down. Rspd. in 1x PBS to 300ml in bag.

Ran through column:

Spun down unabsorbed fraction for 2nd ab incubation.

Spun stem cell fraction to Rspd in smaller volume for count.

Counts:

unabsorbed

mono 67 ✓ poly 102

169 x 50 x 10^3
= 8.5 x 10^6 c/ml x 225ml
= 1.9 x 10^9 C

stem

mono 172 ✓ poly 16

188 x 2 x 10^4
= 3.8 x 10^6 c/ml x 5.5ml
= 20.7 x 10^6 C

incubated unadsorbed fraction w/ 4.5 mL
12.8 ab. for 25 min.
Spun down.
Rst in 300 ml in bag (w/ 1x PBS)
Ran through 2nd column.

Counts:

unadsorbed		stem	
mono	poly	mono	poly
30	33	58	4

$63 \times 53 \times 10^3$
3.15×10^4 cfu/ml x 60 ml
$= 1.9 \times 10^9$ C

$62 \times 2 \times 10^4$
$= 1.2 \times 10^6$ C/ml
$\times 5$ ml $= 6 \times 10^6$ C

⇓

Percolled/ficolled
free ⇒ LOT(2)

combined stem cell fractions

26×10^6 C for transduction
want final [] = 5×10^4 C/ml ∴ 520 ml total
÷ 2 = 260 ml supe
260 ml media

13 flasks 40 ml/flask 20 ml supe
20 ml media (B365)
+ 300 ul protamine sulfate
1:20

LASN supe 539 (bottles 18/19)

CFUs:

SCT145

PRE

Plate #	Sample	# cells	# ul
	(-G418)		
1ab	~~PRE trans~~	5×10^1	5
2ab	↓ (+G418)	↓	5

BBMM + 3116SCF:

1L3 Sandoz # yo230392

1L6 Sandoz # yo150392

SCF AMGEN # 150952

Tocksample to micro for sterility ✓
each day of transduction pd. 0
Stat Gram stain done (negative)
before cells were given to baby

5/15/93 4pm 2nd transduction:
Spun cells down from each flask
Rspd in fresh media & LASN supe
added Protamine sulfate

5/16/93 3rd transduction 330pm.
Repeated above.

# ml	
5	
5	

5/17/93 cells washed 4x
3x in 1x PBS + P/S
last wash in RPMI (no p/s)

count: 60 x 10^6 c $\bar{x}$ = 15 x 10 x 10^4 = 1.5x10^6 c
x 40 ml = 60x10^6 c

Put in 5cc into 10cc syringe

Reinfused on 5/17/93 (UCSF)

Post trans. CFUs: 5/17/46

	sample plate #	# cells	# ml
ØG418	1ab	500	4
↓	2ab	1000	8
↓	3ab	2000	16
+G418	4ab	500	4
↓	5ab	1000	8
↓	6ab	2000	16
ØG418	7ab	1000	24
+G418	8ab	1000	24

y ✓
utive)
) baby
tion
h flask
SN supe
:30pm